

Response of *Pinus koraiensis* seedling growth to different light conditions based on the assessment of photosynthesis in current and one-year-old needles

Jiaojun Zhu • Kai Wang • Yirong Sun • Qiaoling Yan

Received: 2012-11-11;

Accepted: 2012-12-10

© Northeast Forestry University and Springer-Verlag Berlin Heidelberg 2014

Abstract: As one of the three major five-leaved pines in the northern hemisphere, *Pinus koraiensis* is the most important dominant tree species in the natural mixed-broadleaved Korean pine forests. However, the regeneration of *P. koraiensis* under the canopy of secondary forest stands is poor because of the light limitation. This study was conducted to understand how *P. koraiensis* seedlings adapt to different light intensities and what would be the optimum light level for their establishment and growth. Three repetition plots with four light intensities (15%, 30%, 60% and 100% of the natural incident irradiances, achieved by suspending layers of black nylon net above and surrounding the plots) were set up under natural climate conditions in a montane region in eastern Liaoning Province, Northeast China. A total of 80 *P. koraiensis* seedlings with similar height and root collar diameter were transplanted into four plots. After one year of acclimation to the specific light conditions, the seasonal variations of the photosynthetic variables and needle traits of the current and one-year-old needles, and the growth parameters were observed under four light intensities. The results indicated that: (1) The seedling at 60% treatment exhibited the greatest growth, which agreed with the response of the light-saturated photosynthetic rates (A_{\max}) and the dark respiration rate (R_d) in the current and one-year-old needles, i.e., R_d at 60% treatment was significantly lower than that at 100% treatment, but A_{\max} did not differ between the seedlings at 100% and 60% treatments. (2) The *P. koraiensis* seedlings have a certain photosynthetic plasticity to adapt the light conditions by adjusting their needle traits and regulating

the physiological processes, because A_{\max} , R_d , light saturation point and compensation point, the needle mass area, needle nitrogen and chlorophyll contents were significantly ($p < 0.05$) correlated with the light intensities. Especially, A_{\max} at 100% and 60% treatments was significantly higher ($p < 0.05$) than that at 30% and 15% treatments for both current and one-year-old needles. (3) The needles of different ages played a commutative role during the growing season, i.e., the one-year-old needles played a major role for the photosynthesis in the early growing season; the current year needles did in the later growing season. This ensured the effective photosynthesis throughout the growing season. These findings suggest that *P. koraiensis* is the in-between heliophilous and shade-tolerant tree species at least for the seedlings up to 8 years.

Keywords: light requirement, needle age, needle trait, photosynthetic plasticity, *Pinus koraiensis*

Introduction

As one of the three major five-leaved pines in the northern hemisphere, *Pinus koraiensis* Sieb. et Zucc (Korean pine) is the dominant tree species in the natural mixed-broadleaved Korean pine forests (MBKPFs) (the other two five-leaved pine species: *P. sibirica* and *P. strobes*) (Ma et al. 1992). It grows in Northeast China, the Far East of Russia and the Korean peninsula (Tao et al. 1988; Zhao et al. 1991; Barnes et al. 1992; Ma et al. 1992). However, after more than a century of timber exploitation, MBKPFs have greatly declined in both area and number (Chen et al. 2002; Zhu et al. 2007; Li et al. 2012). Almost all of the MBKPFs had been turned into secondary mixed-broadleaved forests or converted to larch (*Larix* spp.) plantations in recent decades (Zhu et al. 2007). This conversion has resulted in inevitable changes in the habitat, such as light, water, and biogeochemical processes of the forest ecosystems in the region (Liu et al. 1998), causing concerns for the long-term fate of the MBKPFs. During recent years, considerable efforts have been made to preserve *P. koraiensis* as a valuable tree species, and the

Fund projection: This research was supported by a grant from the National Nature Science Foundation of China (30830085, 31330016).

The online version is available at <http://www.springerlink.com>

Jiaojun Zhu¹ (✉) • Kai Wang^{1,2} • Yirong Sun^{1,2} • Qiaoling Yan

¹ State Key Laboratory of Forest and Soil Ecology, Qingyuan Experimental Station of Forest Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China; ² University of Chinese Academy of Sciences, Beijing 100039, China.

E-mail: jiaojunzhu@iae.ac.cn;

Corresponding editor: Hu Yanbo

restoration of the MBKPFs is considered as an important measure in maintaining and improving forest ecosystem functions in Northeast China (Li 2004; Zhu et al. 2007; Li et al. 2012).

Light plays a determinant role in the survival and growth of seedlings for many forest tree species that occur beneath the forest canopy (Zhu et al. 2003; Mason et al. 2004; Modrý et al. 2004). As light environments vary greatly during forest stand development and succession at both temporal and spatial scales (Messier and Puttonen 1995), tree species adaptation to the prevailing light conditions is crucial to the successful natural regeneration. Some shade-tolerant tree species can regenerate under almost continuous canopy covers, such as *Picea abies*, *Abies alba* and *Prunus turneriana* (Grassi and Bagnaresi 2001; Bloor and Grubb 2003), whereas the creation of canopy gaps is necessary for the establishment of light-demanding tree species, such as *Pinus sylvestris* and *Populus tremuloides* (Kubiske and Pregitzer 1996; Luoma 1997; Pothier and Prévost 2002). The identification of light requirements is necessary to understanding the regeneration strategy of some tree species and for developing management practices to facilitate natural forest regeneration on environmentally constrained sites.

However, due to the lack of a comprehensive understanding of the light requirements for the establishment of *P. koraiensis* seedlings, artificially facilitated regeneration of the tree species in the secondary broadleaved forests has been mostly fraught with failure (Zhang et al. 2013). Up to now, there has been a contradictory view on the light requirements of *P. koraiensis* seedlings (Wang et al. 2006). Yao et al. (1980) reported that *P. koraiensis* seedlings were light-demanding, and that the biomass of the seedlings reached a maximum under full light condition. But, Wu and Han (1992) found that *P. koraiensis* seedlings were shade tolerant and could survive and grow slowly for a long time under a dense canopy. Therefore, the light requirements of *P. koraiensis* exists uncertainty as its sciophyte or heliophyte.

In order to make clear the light requirements of *P. koraiensis*, we observed the photosynthesis of *P. koraiensis* seedlings in current and one-year-old needles and the growth of the seedlings under different light intensities in a forest area of Northeast China. The objectives of the study were: (1) to examine the photosynthetic plasticity of *P. koraiensis* seedlings to different light intensities; (2) to identify the roles of the current and one-year-old needles of Korean pine in the adaptation to light levels; and (3) to check whether *P. koraiensis* seedlings have an optimum light level for seedling establishment and growth. We studied several photosynthetic variables, needle traits and biomass in the current and one-year-old needles of six-year-old seedlings grown at 15%, 30%, 60% and 100% of the full light.

Materials and methods

Site description

The study was conducted at the Qingyuan Experimental Station of Forest Ecology (QESFE), Chinese Academy of Sciences, in a montane region in eastern Liaoning Province, Northeast China

(41°51'N, 124°54'E, 500–1100 m above sea level). The climate is continental monsoon, with a strong windy spring, a warm and humid summer, and a dry and cold winter. The long-term mean annual air temperature is 4.7°C, with an annual minimum of -37.6°C and an annual maximum of 36.5°C. The mean precipitation is 810.9 mm per annum. The frost-free period is about 130 days, with the first frost occurring in the start of October and the last in the end of April.

Experimental design and treatments

A total of 80 *P. koraiensis* seedlings with similar height and root collar diameter from the same nursery were used in this study. The seedlings had an average height of 16.5±3.3 cm and an average root collar diameter of 0.6±0.1 cm in May of 2007 when the first set of measurements was collected. They were raised in a nursery and transplanted into eight plots with different levels of shading treatment at an age of 5 years in May, 2007. Each plot was set up in a concrete box with 7.5 m long × 2 m wide in dimension and contained top-layer forest soil to a depth of 1 m. There were four levels of light treatments (each light treatment contained two replications or plots): 100% of full light (L-100), 60% of full light (L-60), 30% of full light (L-30), and 15% of full light (L-15). The derived levels of light were achieved by suspending layers of black nylon net above, and surrounding each plot. After one year of acclimation to a specific light condition, the measurements of the photosynthetic variables and needle traits were made in 2008. In order to exclude the effects of other environmental factors, the seedlings were watered in the dry season. There was no fertilizer application. The mean values of the major environmental factors, such as the light intensity i.e., the photosynthetic photon flux density (PPFD), temperature and air humidity, for the four light treatments were measured during the study period in 2008 (Table 1).

Table 1. Average values (mean ± SE) of environmental factors within the observation months (July–October) in 2008

Light levels	Mean photosynthetic photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Air temperature (°C)	Air relative humidity (%)
L-100	842.4±20.00 ^d	26.8±0.13 ^a	35.37±2.70 ^b
L-60	544.9±16.71 ^c	26.0±0.13 ^b	40.16±2.77 ^a
L-30	263.2±5.32 ^b	25.8±0.17 ^b	39.53±2.59 ^a
L-15	125.3±2.73 ^a	25.9±0.16 ^b	39.59±2.54 ^a

Note: data in the column with different letters indicate the significant difference at $p < 0.05$. The environmental factors were recorded during the observation period of photosynthesis.

Measurements of the photosynthetic parameters

During the growing season from May to October, photosynthetic light-response curves were developed by measuring the photosynthesis rates in the current and one-year-old needles at 11 levels of the PPFD (starting from 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, then 20, 50, 100, 200, 400, 600, 800, 1000, 1500 and 2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) under ambient CO₂ concentrations (380 $\mu\text{mol}\cdot\text{mol}^{-1}$) with a portable

photosynthesis system (LI-6400, LiCor, Lincoln, NE, USA). The light-saturated photosynthetic rate (A_{\max}), dark respiration rate (R_d), apparent quantum yield (AQY), light compensation point (LCP) and light saturation point (LSP) were derived by fitting the photosynthetic light-response curve (Farquhar and Sharkey 1982). The curves of the photosynthetic response to intercellular CO_2 concentration (A/C_i) were developed by measuring the rate of photosynthesis at ten CO_2 concentrations, beginning at 400, then sequentially at 300, 200, 100, 50, 400, 600, 800, 1000 and 1500 $\mu\text{mol}\cdot\text{mol}^{-1}$, at variable light-saturated PPFDs, depending on the growth light environments of the measured seedlings (i.e., 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in L-100, 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in L-60, 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in L-30, and 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in L-15) with the artificial LI-6400-02B light source. The leaf chamber was set at a temperature of 25°C and a relative humidity of 50% during the measurements. The ribulose-1, 5-bisphosphate (RuBP) carboxylation capacity (V_{\max}) and the maximum rate of electron transport (J_{\max}) were estimated from the A/C_i curves fit by nonlinear regression, based on the model of Farquhar and Sharkey (1982).

The measurements of photosynthesis were made on healthy, upper-crown needles of different ages, and were repeated on three branches, from three individual seedlings in the plots of each treatment. All of the measurements were made in the morning, between 8:30 and 11:00 a.m. (GMT+008) on sunny days for 10 days of each month. The values of the net photosynthetic rate were expressed on a leaf-area basis, and the width and length of needles were measured by Vernier calipers for calculating the total surface area of the needles used in each set of photosynthesis measurements (Choi et al. 2006). The photosynthetic parameters of the current-year needles were measured from July, when those needles reached full expansion. All the data presented are averaged to get a single value for each light treatment.

Leaf mass area, nitrogen content, and chlorophyll content of the needles

The needles used in the gas exchange measurements were dried at 80°C for 72 hours after the determination of the surface area and then calculated for the leaf mass area (LMA, defined as the ratio of leaf dry mass to leaf surface area, $\text{g}\cdot\text{m}^{-2}$) (Díaz-Barradas et al. 2010). The leaf chlorophyll (Chl) content was determined spectrophotometrically using 80% acetone extracts (Díaz-Barradas et al. 2010). The total leaf nitrogen (N) contents were determined on the oven-dried samples using an elemental analyzer (Vario EL III, Germany).

Growth parameters

The root collar diameter and height of the experiment seedlings were measured at the end of October. Three average seedlings (7 years old) in each plot were harvested at the end of the experiment in 2009. The number of needles, aboveground biomass (needle and stem separately) and belowground biomass were measured. The dry mass of each component (needle, stem and root) of the seedlings was obtained after the samples were dried at 80°C for 72 hours.

Statistical analysis

The data of photosynthetic variables presented in the figures were averaged with the three repetitions in the observation month. The effects of the light levels and months on the photosynthetic variables as the function of the needle ages were evaluated by two-way repeated-measures ANOVA. The least significant difference (LSD) was used to differentiate the means among the light levels and months. Student's *t*-test was used for the pairwise comparisons between the current and one-year-old needles at the same light level. The differences between treatments in the photosynthetic variables, leaf traits and biomass were reported as significant when $p < 0.05$. The relationships of the light levels with photosynthetic variables and leaf traits were examined by linear regression analysis. All of the statistical tests were performed using SPSS 13.0.

Results

Variations of the photosynthetic variables with the light levels and months

All of the photosynthetic variables except AQY were significantly influenced by the light levels for both the current and one-year-old needles ($p < 0.05$) (Table 2). Months had no significant effects on LSP and LCP of the current-year needles, and on LSP and V_{\max} of the one-year-old needles ($p > 0.05$) (Table 2).

The A_{\max} had maximum values under the L-60 light level in the current-year needles, except for the first month of observations (July) (Fig. 1A). In the one-year-old needles, the maximum values of A_{\max} appeared under the L-100 light level during the first three months of observations (i.e., June, July and August); whereas, during the later two months (September and October) the maximum values of A_{\max} occurred under the L-60 and L-30 light levels (Fig. 1B). The A_{\max} exhibited less fluctuation under L-30 and L-15 light levels for both the current and one-year-old needles during the observation months (Fig. 1A, B).

Most of the R_d of both the current and one-year-old needles increased with the increase of the light levels from L-30 to L-100 during the observation period (exception for July in current-year needles and June in one-year-old needles) (Fig. 1C, D). The mean R_d of L-100 treatment in current-year needles was significantly higher than that of L-60 treatment ($p < 0.05$) (Fig. 1D). Similar to A_{\max} , the R_d of the current-year needles exhibited less fluctuation under L-30 and L-15 light levels (Fig. 1C). While, the R_d of one-year-old needles did not show the similar trend to those of the current-year needles under L-30 and L-15 light levels (Fig. 1D). The AQY decreased from August to October in the L-100 and L-60 treatments for the current-year needles and in the L-100 treatment for the one-year-old needles, whereas it remained constant in the seedlings subjected to the other light treatments (Fig. 1E, F). There were significant monthly variations in the A_{\max} and R_d for the current-year needles in the L-100

and L-60 treatments and for the one-year-old needles in the L-100 treatment (Fig. 1).

Table 2. Results of two-way repeated-measures ANOVA showing the *P* values for photosynthetic variables and needle traits in the current and one-year-old needles of *Pinus koraiensis* seedlings combined with light and month as main factors.

Needle age	Factors	A_{\max}	R_d	AQY	V_{\max}	J_{\max}	LSP	LCP	LMA	N	Chl
Current -year	Light	0.001	0.000	0.473	0.000	0.003	0.000	0.000	0.000	0.000	0.000
	Month	0.045	0.002	0.015	0.001	0.000	0.112	0.252	0.000	0.000	0.000
One-year-old	Light	0.001	0.001	0.172	0.000	0.003	0.000	0.000	0.000	0.000	0.000
	Month	0.017	0.001	0.001	0.055	0.006	0.373	0.039	0.000	0.000	0.000

Note: A_{\max} : light-saturated photosynthetic rate, R_d : dark respiration rate, AQY: apparent quantum yield, V_{\max} : ribulose-1, 5-bisphosphate carboxylation capacity, J_{\max} : the maximum rate of electron transportsion, LSP: light saturation point, LCP: light compensation point, LMA: leaf mass area, Chl: leaf chlorophyll content, N: total leaf nitrogen content.

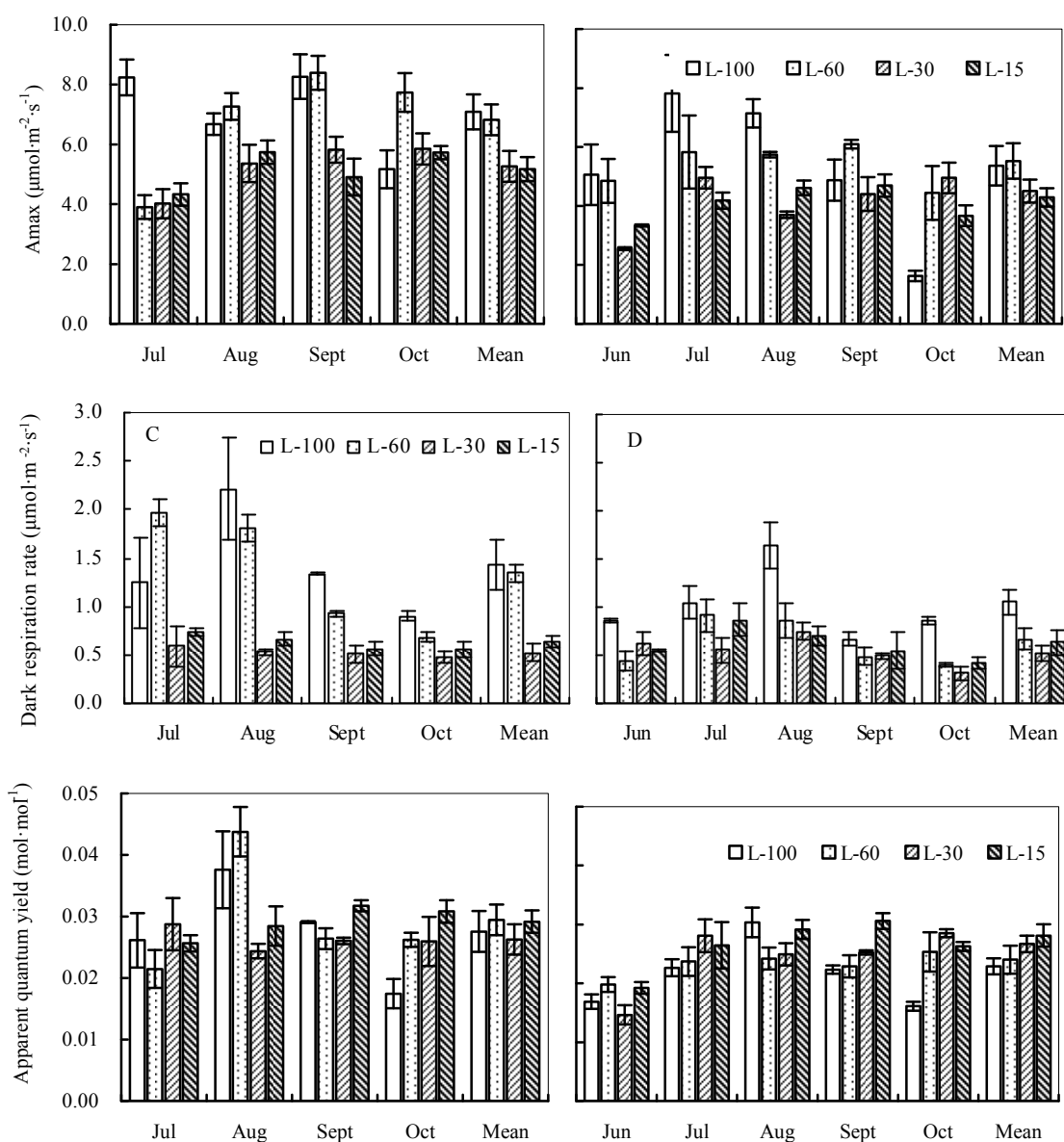


Fig. 1. Seasonal variations in light-saturated photosynthetic rate (A_{\max}), dark respiration rate (R_d) and apparent quantum yield (AQY) of *Pinus koraiensis* seedlings from June (for the one-year-old needles) or July (for the current-year-old needles) to October. A, C & E: the current-year needles; B, D & F: the one-year-old needles. Values are means \pm SE of the three replicated needles.

The V_{cmax} increased from July through September and then decreased in October for the current-year needles under L-60 and L-30 light levels (Fig. 2A). While, the V_{cmax} for the one-year-old needles did not show the similar trend to that of the current-year needles. The mean V_{cmax} of one-year-old needles during the observation period (June–October) ranked as: L-60 > L-100 > L-30 > L-15 (Fig. 2B). The J_{max} increased from the early-season

measurements to the end of the experiment in the current-year needles for all the four light levels (Fig. 2C). But the J_{max} for the one-year-old needles did not exhibit the similar trend to that of the current-year needles, it showed the similar patterns of the mean V_{cmax} for the one-year-old needles (L-60 > L-100 > L-30 > L-15) (Fig. 2D).

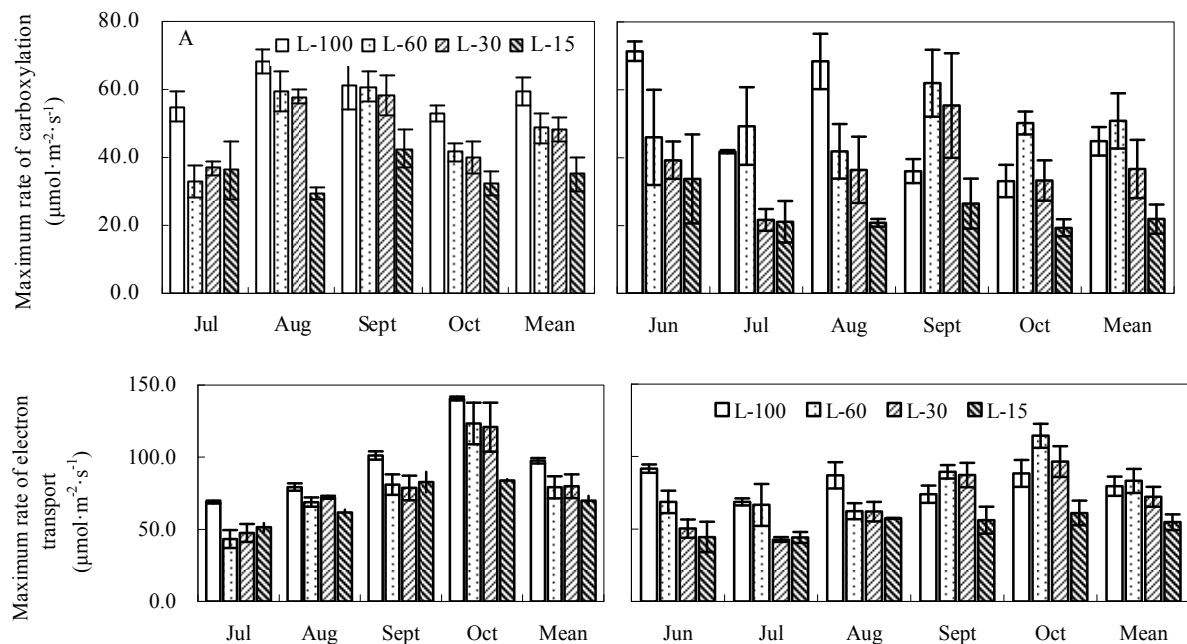


Fig. 2. Seasonal variations in maximum rate of carboxylation (V_{cmax}) and maximum rate of electron transport (J_{max}) of *Pinus koraiensis* seedlings from July to October. A & C: the current-year needles; B & D: the one-year-old needles. Values are means \pm SE of three replicate needles.

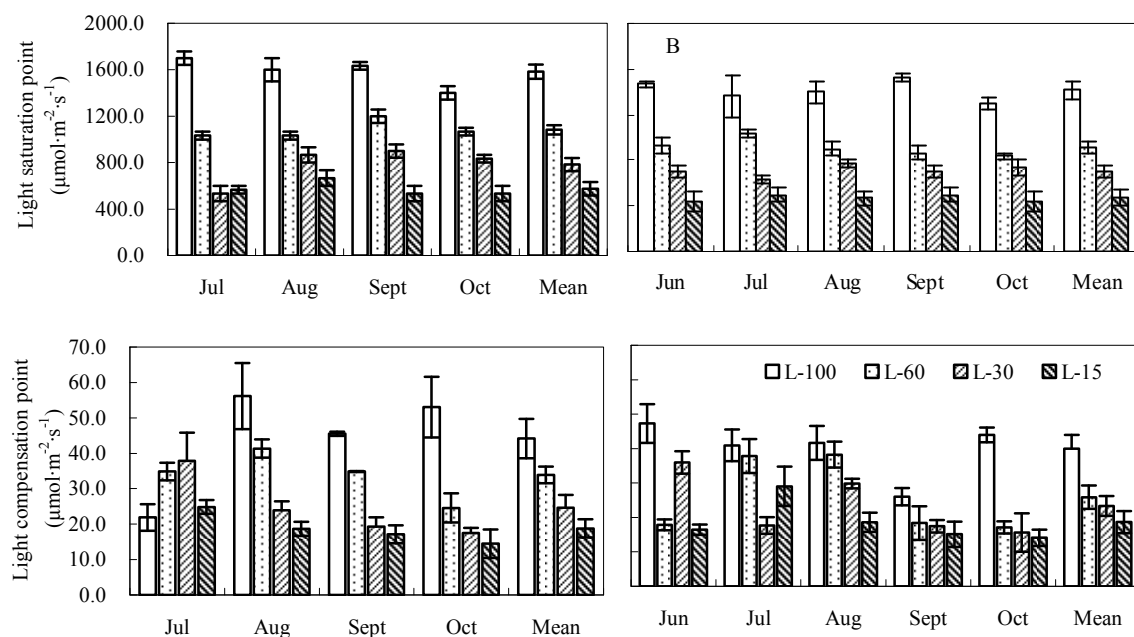


Fig. 3. Seasonal variations in light saturation point (LSP) and light compensation point (LCP) of *Pinus koraiensis* seedlings from July to October. A & C: the current-year needles; B & D: the one-year-old needles. Values are means \pm SE of three replicate needles.

The LSP significantly increased with the light levels in both the current and one-year-old needles (Fig. 3A, B). But the LCP did not exhibit the clear increasing patterns compared to LSP (Fig. 3C, D).

Variations of the needle traits with the light levels and months

There were significant differences between the current and one-year-old needles in the monthly patterns of the LMA, needle N, and Chl (Fig. 4, Table 3). Generally, the LMA, needle N, and

Chl in the current-year needles increased with time throughout the experiment ($p < 0.05$), whereas they did not display significant monthly changes in the one-year-old needles ($p > 0.05$). The LMA, needle N and Chl increased with the light levels: they were greater in the L-100 and L-60 treatments than in the L-30 and L-15 treatments in both the current and one-year-old needles (Fig. 4). On average, the LMA, needle N, and Chl in the current-year needles were respectively 10.5%, 20.5%, and 2.25% greater than in the one-year-old needles.

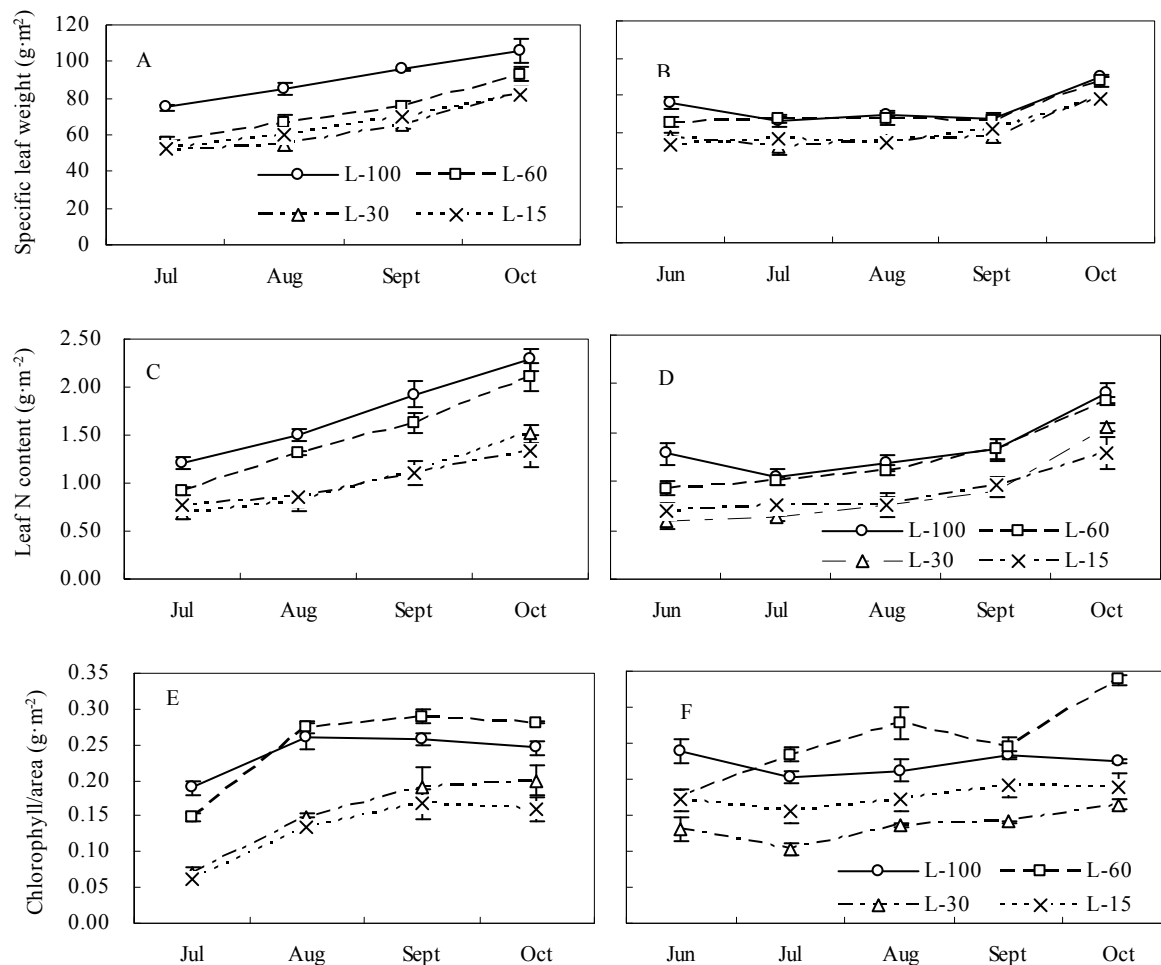


Fig. 4. Seasonal variations in leaf mass area (LMA), leaf N content (N) and chlorophyll content (Chl) of *Pinus koraiensis* seedlings from July to October. A, C & E: the current-year needles; B, D & F: the one-year-old needles. Values are means \pm SE of three replicate needles.

Relationships between the photosynthetic variables and needle traits

When the data for each age class and each light treatment were analyzed separately, significantly positive relationships of A_{\max} -LMA, A_{\max} -N, and A_{\max} -Chl were found for the current-year needles in the L-60, L-30, and L-15 treatments ($p < 0.05$), whereas the A_{\max} was strongly correlated with the stomatal conductance (g_s) for the one-year-old needles across all of the light treatments ($p < 0.05$, Table 3). Positive correlations of J_{\max} -LMA, J_{\max} -N, and

J_{\max} -Chl were found for both the current and one-year-old needles in all the four light treatments (exception for J_{\max} -Chl in L-100 treatment) ($p < 0.05$, Table 3). Almost non-significant correlations were found between V_{\max} and the needle traits (LMA, N and Chl), and g_s for all the light treatments ($p > 0.05$, Table 3). When the data from all of the treatments were pooled, the needle N content was closely related to the LMA in both the current and one-year-old needles (current-year: $r = 0.94$, $p < 0.05$; one-year-old: $r = 0.93$, $p < 0.05$). The V_{\max} was linearly related to the J_{\max} for the one-year-old needles ($r = 0.69$, $p < 0.05$).

Seedling growth parameters

The growth in height did not exhibit a significant difference between the light levels after one year of light acclimation (Fig. 5A). However, significant differences were found between the treatments of L-100 or L-60 and the treatments of L-30 or L-15 after two years of light acclimation and among the L-100, L-60, and the L-30 or L-15 after three years of light acclimation ($p<0.05$) (Fig. 5A). The root collar diameter growth showed similar trends (Fig. 5B), although a significant difference was found between the L-100 and L-60 treatments after two years of light acclimation. We observed the same trends of biomass in

response to the light levels for the aboveground (needle, stem or branch), belowground and total biomass (Fig. 6A, B, C), i.e., the biomass in the L-60 treatment was significantly higher than in the other treatments. However, there was no significant difference of stem biomass between the L-60 and L-100 treatments (Fig. 6A). For the increase in the biomass, there was no significant difference between the L-30 and L-15 treatments ($p>0.05$); and it was significantly higher under L-100 treatment than that of the L-30 and L-15 treatments. The ratios of above ground mass (AGM) to underground mass (UGM) showed no significant difference among the different light levels; but the ratio of needle mass to AGM exhibited significantly lower in L-100 treatment (Fig. 6D).

Table 3. Correlation coefficients (r) with significance level of linear regression for light-saturated photosynthetic rate (A_{\max}), maximum rates of carboxylation (V_{\max}) and electron transport (J_{\max}) with leaf mass area (LMA), leaf N content (N), leaf chlorophyll content (Chl), and stomatal conductance to water vapor (g_s) in the current and one-year-old needles of *Pinus koraiensis* seedlings grown at L-100, L-60, L-30, and L-15.

Factors	Age class	Light level	LMA	N	Chl	g_s
A_{\max}	Current-year	L-100	-0.3481 ^{ns}	-0.3360 ^{ns}	-0.0426 ^{ns}	0.8067*
		L-60	0.7565*	0.7919*	0.9497*	0.725*
		L-30	0.6952*	0.7157*	0.8462*	0.0761*
		L-15	0.5870*	0.616*	0.7029*	0.0309 ^{ns}
	One-year-old	L-100	-0.582*	-0.597*	-0.0558 ^{ns}	0.9045*
		L-60	-0.1006 ^{ns}	-0.0916 ^{ns}	0.0222 ^{ns}	0.9107*
		L-30	0.3887 ^{ns}	0.4651 ^{ns}	0.1684 ^{ns}	0.6986*
		L-15	0.0469 ^{ns}	0.1926 ^{ns}	0.3632 ^{ns}	0.7504*
J_{\max}	Current-year	L-100	0.8982*	0.9439*	0.4024 ^{ns}	-0.8277*
		L-60	0.9598*	0.9813*	0.6896*	0.3318 ^{ns}
		L-30	0.8972*	0.9162*	0.8395*	-0.6376*
		L-15	0.8981*	0.8458*	0.8959*	-0.7150*
	One-year-old	L-100	0.5847*	0.5698*	0.6101*	-0.4672 ^{ns}
		L-60	0.8240*	0.8688*	0.6717*	-0.3325 ^{ns}
		L-30	0.7360*	0.8432*	0.8376*	-0.1862 ^{ns}
		L-15	0.6808*	0.6245*	0.6412*	0.1070 ^{ns}
V_{\max}	Current-year	L-100	-0.0196 ^{ns}	0.0199 ^{ns}	0.6251*	-0.0697 ^{ns}
		L-60	0.2591 ^{ns}	0.2718 ^{ns}	0.7388*	0.2853 ^{ns}
		L-30	-0.0179 ^{ns}	0.0613 ^{ns}	0.5474 ^{ns}	-0.1351 ^{ns}
		L-15	0.2047 ^{ns}	0.4028 ^{ns}	0.3508 ^{ns}	0.0561 ^{ns}
	One-year-old	L-100	-0.1138 ^{ns}	-0.2608 ^{ns}	0.2739 ^{ns}	0.1523 ^{ns}
		L-60	0.3062 ^{ns}	0.3338 ^{ns}	0.2331 ^{ns}	0.1101 ^{ns}
		L-30	0.1882 ^{ns}	0.1826 ^{ns}	0.4390 ^{ns}	-0.3557 ^{ns}
		L-15	0.1164 ^{ns}	0.1005 ^{ns}	0.2451 ^{ns}	-0.1302 ^{ns}

The significance indicates as: * represents $p<0.05$, and ^{ns} represents $p\geq0.05$.

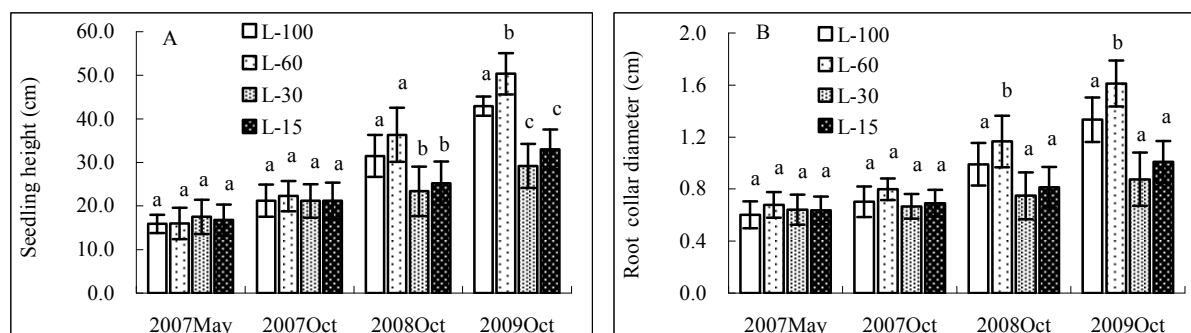


Fig. 5. Seedling height (A) and root collar diameter (B) under different light levels at the end of the experiment. Different letters above the histogram indicate significant differences between light treatments at $p<0.05$.

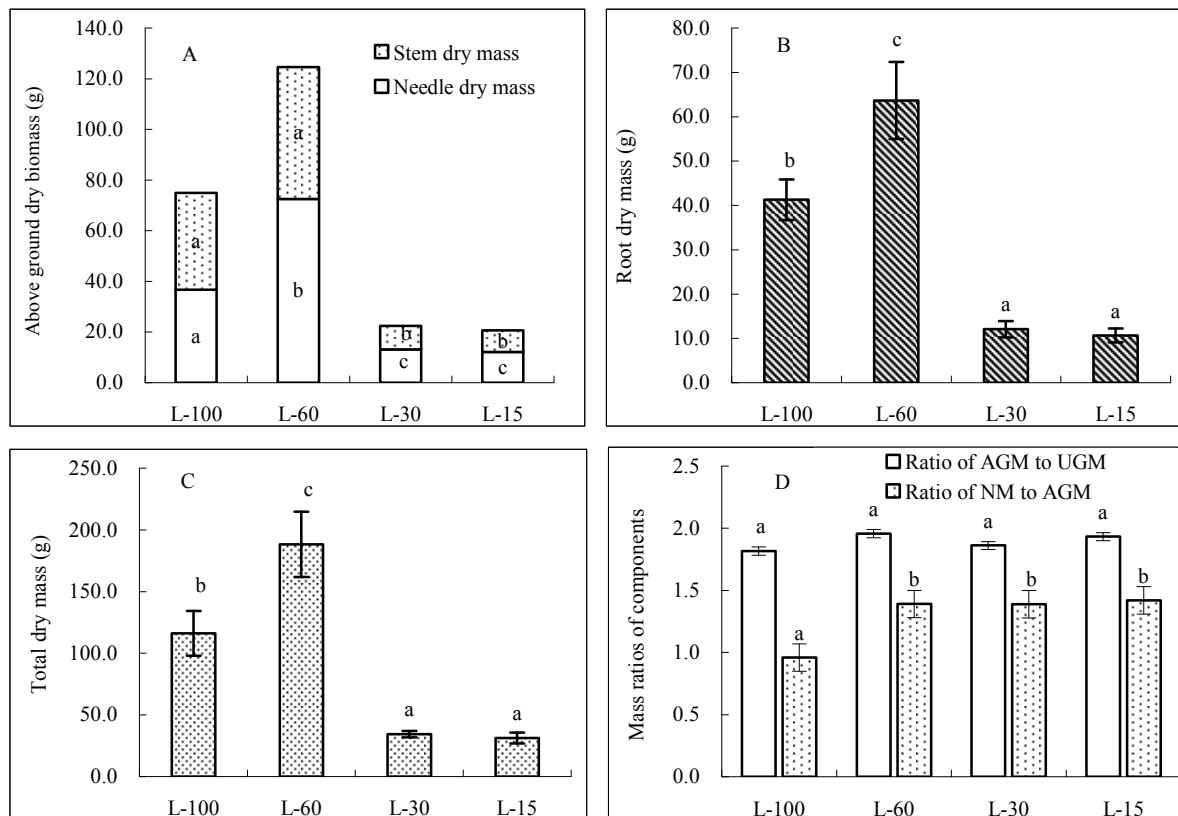


Fig. 6. Biomass of seedlings under different light levels at the end of the experiment; aboveground biomass in stem and needle separately (A), underground biomass (B), total biomass (C), and the ratios of above ground mass (AGM) to underground mass (UGM) and the needle mass (NM) to AGM (D). Different letters above or on the histogram indicate significant differences between light treatments at $p < 0.05$.

Discussion

Photosynthetic plasticity of the *P. koraiensis* seedlings based on the response of the photosynthetic variables to light levels

The A_{\max} exhibited the maximum values under the L-100 and L-60 light levels in both the current and one-year old needles during the observation, indicating that the full light and 60% full light may be the better light levels for the photosynthesis of the *P. koraiensis* seedlings. The exception of A_{\max} in the current-year needles in July (the first month of the observation) was induced because the current-year needles did not develop completely in July. The values of R_d in the current-year needles were significantly lower, under L-15, L-30 and L-60 treatments, than those under L-100 treatment, which can be explained as a strategy of energy conservation by the seedlings to minimize the respiratory loss of CO_2 in order to produce a positive carbon balance under the low light conditions. These results implied that needles under L-60 treatment might accumulate more biomass than that under L-100 treatment because no significant difference of A_{\max} between L-100 and L-60 treatments, but significantly lower R_d in

L-60 treatment.

Generally, the LCP and LSP are both good indicators of light requirements, with sciophytes normally exhibiting lower values than heliophytes (Griffin et al. 2004; Pastur et al. 2007); in addition, the R_d and AQY in shade-tolerant species tend to be lower than in shade-intolerant species (Bond et al. 1999; Pothier and Prévost 2002). We found that the *P. koraiensis* seedlings had lower LCP and LSP values in response to decreasing light levels for both the current and one-year-old needles, indicating a degree of photosynthetic plasticity to the varying light conditions. This suggests that the variations in physiological processes in terms of the R_d , LSP, and LCP allow *P. koraiensis* seedlings to tolerate a wide range of light environments for survivals. The AQY was lower after August, under both L-100 and L-60 light levels for the current-year needles and under L-100 light level for the one-year-old needles of the *P. koraiensis* seedlings, indicating an apparent photoinhibition to avoid damage to the photosynthetic apparatus by excessive energy (Boardman 1977). We found there was a stronger relationship in the current-year needles between the light levels and needle traits (i.e., the LMA and N and Chl contents), indicating that the capability of acclimation to the light conditions was closely related to the growth stage of the needles. The better acclimation of the current-year needles could have,

perhaps, compensated for the lower photosynthetic ability (A_{\max}) of the one-year-old needles in the later part of the growing season (Fig. 1A, B).

By comparing the values of the A_{\max} , R_d , AQY, LCP and LSP observed in the *P. koraiensis* seedlings under various light levels with those of light demanding and shade-tolerant tree species, we found that all of the values fell between those of the heliophilous and shade-tolerant tree species. For example, the mean value of the A_{\max} under the different light levels varied between 4.10 and 7.09 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the *P. koraiensis* seedlings, which was in a range between the values of the sciophytes, *Abies balsamea* (4-year old seedlings, 4 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Kayama et al. 2002; Pothier and Prévost 2002), and those of the heliophyte, *P. sylvestris* (18-year old saplings, 8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Luoma 1997). The mean value of the R_d under the different light levels varied between 0.53 and 1.43 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the AQY varied between 0.023 and 0.029 $\text{mol CO}_2\cdot\text{mol}^{-1}$, which were higher than the sciophyte, 4-year old seedlings of *A. balsamea* ($R_d = 0.05 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $\text{AQY} = 0.02 \text{ mol CO}_2\cdot\text{mol}^{-1}$), and lower than the heliophyte, 4-year old seedlings of *Populus tremuloides* ($R_d = 2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $\text{AQY} = 0.04 \text{ mol CO}_2\cdot\text{mol}^{-1}$) (Pothier and Prévost 2002). The same trends were observed for the LSP and LCP (i.e., the LSP varied between 467 and 1583 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and the LCP between 18.8 and 44.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively) of the *P. koraiensis* seedlings, which were greater than those in the sciophyte, 4-year old seedlings of *Picea abies* (LSP $\approx 450 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and LCP $\approx 10.0\text{--}20.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Grassi and Bagnaresi 2001; Kayama et al. 2002), and lower than in the heliophytes, 18-year old saplings of *P. sylvestris* (LSP $\approx 1700 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, LCP $\approx 70.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Luoma 1997). Therefore, *P. koraiensis*, at least for 6- or 7-year-old seedlings, could be considered as a tree species that lies in the range between the heliophilous and shade-tolerance species. The result of this study demonstrated the photosynthetic plasticity of *P. koraiensis* seedlings to varying light conditions, which is similar to the other five-needle pines (*P. sibirica*, seven to ten years old seedlings; and *P. strobes*, eight years old seedlings) (Mudrik and Vil'chek 2001; Major et al. 2009).

Effects of light levels on the *P. koraiensis* seedling growth

There was a clear trend in seedling growth, with the greatest height and the largest root collar diameter under the L-60 treatment at the end of the experiment. The same trend was found with regards to the biomass parameters. The growth response of the *P. koraiensis* seedlings to the different light levels may be explained by the photosynthetic variables observed in the needles of different ages.

In general, the light-saturated photosynthetic rate (A_{\max}) is related to the developmental stage of the leaves, such that when they are in the process of unfolding or when they are not fully expanded, the leaves do not have fully active chloroplasts; conversely, well-developed foliage is at the peak of its photosynthetic performance (Hieke et al. 2002; Pastur et al. 2007). In our experiment, photosynthetic measurements were not conducted in the current-year needles during June because the current-year

needles were not well developed. However, the average values of the A_{\max} for the current-year needles were 32.1%, 23.5%, 16.9% and 20.7% greater than those for the one-year-old needles during July and October under the L-100, L-60, L-30, and L-15 light levels, respectively. This result was in agreement with the greater N content in the current-year needles, as compared to the one-year-old needles (Fig. 4). In addition, the highest chlorophyll content in both the current and one-year-old needles was found in the L-60 treatment, which may help to ensure effective photosynthetic rates for the *P. koraiensis* seedlings under such light condition (Pastur et al. 2007). The dark respiration rate (R_d) has been shown to be correlated with plant growth stages (Pastur et al. 2007). In our observations, the R_d values in both the current and one-year-old needles were higher under L-100 treatment than those under L-60 treatment (Fig. 1C, D), which indicated that the *P. koraiensis* seedlings under L-60 treatment consumed less mass than under L-100 treatment because A_{\max} values under both treatments were similar to each other. The V_{cmax} and J_{max} were significantly related to the LMA and the needle N in both the current and one-year-old needles, in agreement with the results of growth data (Figs. 5, 6). These results may have contributed to the overall reduction of the mass accumulation in the one-year-old needles. In addition, the ratio of needle biomass to above ground mass was significantly lower in L-100 light level, indicating that seedlings under L-100 may produce relatively less dry mass even though the A_{\max} value under L-100 was higher (Zhang et al. 2013).

Conclusions

This study shows that the photosynthetic status and needle traits of *P. koraiensis* seedlings can change in response to various light intensities, which may ensure that *P. koraiensis* seedlings are able to grow and survive under different light regimes. The results highlight the photosynthetic plasticity of the *P. koraiensis* seedlings to varying light intensities. This plasticity allowed the *P. koraiensis* seedlings to grow and survive under the condition of 15% of the full light by improving the efficiency of photosynthesis to the relatively low light intensities, and to produce the photoinhibition response to avoid the damage caused by the more intense light levels. The treatment of L-60 resulted in the highest growth, which indicates that the *P. koraiensis* seedlings have an optimum light condition, even though they may exhibit photosynthetic plasticity. These findings suggest that *P. koraiensis* is the in-between heliophilous and shade-tolerant tree species at least for the seedlings up to 8 years. The conclusions are only based on the light quantity without considering light quality although light quality is important for the establishment of *P. koraiensis* seedlings.

Acknowledgments

We appreciate Professor Osbert Jianxin Sun from Beijing Forestry University, China for his careful revision on the manuscript.

References

- Barnes BV, Xu ZB, Zhao SD. 1992. Forest ecosystems in an old-growth pine-mixed hardwood forest of Changbai Shan Preserve in northeastern China. *Canadian Journal of Forest Research*, **22**: 144–160.
- Bloor JMG, Grubb PJ. 2003. Growth and mortality in high and low light: trends among 15 shade-tolerant tropical rain forest tree species. *Journal of Ecology*, **91**: 77–85.
- Boardman NK. 1977. Comparative photosynthesis of sun and shade plants. *Annual Review Plant Physiology*, **28**: 355–377.
- Bond BJ, Farnsworth BT, Coulombe RA, Winner WE. 1999. Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. *Oecologia*, **120**: 183–192.
- Chen XW, Zhou GS, Zhang XS. 2002. Spatial characteristics and change for tree species along the North East China Transect (NECT). *Plant Ecology*, **164**: 65–74.
- Choi DS, Kayama M, Jin HO, Lee CH, Izuta T, Koike T. 2006. Growth and photosynthetic responses of two pine species (*Pinus koraiensis* and *Pinus rigida*) in a polluted industrial region in Korea. *Environmental Pollution*, **139**: 421–432.
- Díaz-Barradas MC, Zunzunegui M, Ain-Lhout F, Jáuregui J, Boutaleb S, Álvarez-Cansino L, Esquivias MP. 2010. Seasonal physiological responses of *Argania spinosa* tree from Mediterranean to semi-arid climate. *Plant and Soil*, **337**: 217–231.
- Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, **33**: 317–345.
- Grassi G, Bagnaresi U. 2001. Foliar morphological and physiological plasticity in *Picea abies* and *Abies alba* saplings along a natural light gradient. *Tree Physiology*, **21**: 959–967.
- Griffin JJ, Ranney TG, Pharr DM. 2004. Photosynthesis, chlorophyll fluorescence, and carbohydrate content of *Illicium* taxa grown under varied irradiance. *Journal of the American Society for Horticultural Science*, **129**: 46–53.
- Hieck S, Menzel CM, Ludders P. 2002. Effect of leaf, shoot and fruit development on photosynthesis of lychee trees (*Litchi chinensis*). *Tree Physiology*, **22**: 955–961.
- Kayama M, Sasa K, Koike T. 2002. Needle life span, photosynthetic rate and nutrient concentration of *Picea glehnii*, *P. jezoensis* and *P. abies* planted on serpentine soil in northern Japan. *Tree Physiology*, **22**: 707–716.
- Kubiske ME, Pregitzer KS. 1996. Effects of elevated CO₂ and light availability on the photosynthetic light response of trees of contrasting shade tolerance. *Tree Physiology*, **16**: 351–358.
- Li WH. 2004. Degradation and restoration of forest ecosystems in China. *Forest Ecology and Management*, **201**: 33–41.
- Li YB, Mou P, Wang TM, Ge JP. 2012. Evaluation of regeneration potential of *Pinus koraiensis* in mixed pine-hardwood forests in the Xiao Xing'an Mountains, China. *Journal of Forestry Research*, **23**: 543–551.
- Liu SR, Li XM, Niu LM. 1998. The degradation of soil fertility in pure larch plantations in the northeastern part of China. *Ecology Engineering*, **10**: 75–86.
- Luoma S. 1997. Geographical pattern in photosynthetic light response of *Pinus sylvestris* in Europe. *Functional Ecology*, **11**: 273–281.
- Ma JL, Zhuang LW, Chen D. 1992. Geographic distribution of *Pinus koraiensis* in the world. *Journal of Northeast Forestry University*, **20**(5): 40–48 (in Chinese with English abstract).
- Major JE, Mosseler A, Barsi DC, Corriveau-Dupuis B, Campbell M. 2009. Impact of three silvicultural treatments on growth, light-energy processing, and related needle-level adaptive traits of *Pinus strobus* from two regions. *Forest Ecology and Management*, **257**: 168–181.
- Mason WL, Edwards C, Hale SE. 2004. Survival and early seedling growth of conifers with different shade tolerance in a Sitka spruce spacing trial and relationship to understory light climate. *Silva Fennica*, **38**(4): 357–370.
- Messier C, Puttonen P. 1995. Spatial and temporal variation in the light environment of developing Scots pine stands: the basis for a quick and efficient method of characterizing light. *Canadian Journal of Forest Research*, **25**: 343–354.
- Modrý M, Hubený D, Rejšek K. 2004. Differential response of naturally regenerated European shade tolerant tree species to soil type and light availability. *Forest Ecology and Management*, **188**: 185–195.
- Mudrik VA, Vil'chek GE. 2001. Ecophysiological responses of *Larix sibirica* Ledeb. and *Pinus sibirica* Du Tour undergrowth to climate change. *Russian Journal of Ecology*, **32**: 243–248.
- Pastur GM, Lencinas MV, Peri PL. 2007. Photosynthetic plasticity of *Notofagus pumilio* seedlings to light intensity and soil moisture. *Forest Ecology and Management*, **243**: 274–282.
- Pothier D, Prévost M. 2002. Photosynthetic light response and growth analysis of competitive regeneration after partial cutting in a boreal mixed stand. *Trees*, **16**: 365–373.
- Tao DL, Jin YH, Du YJ. 1988. Novel photosynthesis-light curves of solar-exposed versus shaded Korean pine seedling. *Environmental and Experimental Botany*, **28**: 301–305.
- Wang WJ, Zu YG, Wang HM, Matsuura Y, Sasa K, Koike T. 2006. Newly-formed photosynthates and the respiration rate of girdled stems of Korean pine (*Pinus koraiensis* Sieb. et Zucc.). *Photosynthetica*, **44**: 147–150.
- Wu YG, Han JX. 1992. Analysis on the structure of Korean Pine population and its natural regeneration patterns. In: Wang, Z., Li, W.H. (eds), *Research of forest ecosystem*. Beijing: Chinese Forestry Press, pp 14–23. (in Chinese)
- Yao Y, Yang SH, Cao J. 1980. The effect of light intensity on the growth and some physiological activities of Korean Pine. In: Wang, Z., Li, W.H. (eds), *Research of forest ecosystem*. Beijing: Chinese Forestry Press, pp 43–50. (in Chinese)
- Zhao GY, Hao AJ, Yang CT. 1991. Determination about the Northwestern area limit of *Pinus koraiensis* and the geographical occurrence of *Pinus sibirica*. *Journal of Northeast Forestry University*, **2**: 42–46. (in Chinese with English abstract)
- Zhang M, Zhu JJ, Li MC, Zhang GQ, Yan QL. 2013. Different light acclimation strategies of two coexisting tree species seedlings in a temperate secondary forest along five natural light levels. *Forest Ecology and Management*, **306**: 234–242.
- Zhu JJ, Mao ZH, Hu LL, Zhang, JX. 2007. Plant diversity of secondary forests in response to anthropogenic disturbance levels in montane regions of northeastern China. *Journal of Forest Research*, **12**: 403–416.
- Zhu JJ, Matsuzaki T, Li FQ, Gonda Y. 2003. Effect of gap size created by thinning on seedling emergency, survival and establishment in a coastal pine forest. *Forest Ecology and Management*, **182**: 339–354.